

WHAT IS CLAIMED IS:

1. A method of performing a mobility shift assay in a microfluidic device, the method comprising:

5 flowing a reaction mixture comprising an enzyme, an enzyme substrate, and a product through a separation region of the microfluidic device under an applied pressure, which separation region comprises at least one ion-exchange material, to separate the product from at least one other material based upon a net charge difference between the product and the at least one other material to produce separated materials; and,

10 detecting at least one of the separated materials, thereby performing the mobility shift assay in the microfluidic device.

2. The method of claim 1, wherein the at least one other material comprises the enzyme and/or unreacted enzyme substrate.

3. The method of claim 1, wherein at least the separated materials are  
15 flowed in the microfluidic device in an absence of an applied electric field.

4. The method of claim 1, wherein at least the separated materials are flowed in the microfluidic device under at least one simultaneously applied electric field.

5. The method of claim 1, wherein one or more of the separated materials comprise a label.

20 6. The method of claim 1, wherein a microchannel comprises the separation region.

7. The method of claim 1, wherein the applied pressure is produced by a vacuum pump operably connected to the microfluidic device through a port that fluidly communicates with the separation region.

25 8. The method of claim 1, wherein the detecting step comprises at least an optical, a spectroscopic, a fluorescent, a mass, or a luminescent detection.

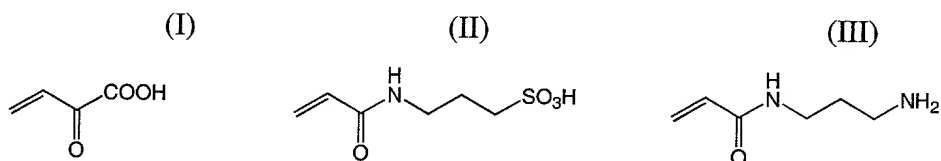
9. The method of claim 1, wherein a plurality of microbeads or a gel comprises the ion-exchange material.

10. The method of claim 1, wherein an inner surface of the separation region comprises the ion-exchange material.

5 11. The method of claim 1, wherein the ion-exchange material is coated on an inner surface of the separation region.

12. The method of claim 1, wherein the ion-exchange material comprises one or more of: a polyarginine, a polylysine, a modified polyacrylamide, or a modified dimethylacrylamide.

10 13. The method of claim 1, wherein the ion-exchange material comprises a polyacrylamide material or a dimethylacrylamide material modified by one or more additives having formula (I), (II), or (III):



15 14. The method of claim 1, further comprising sampling the reaction mixture from a source external to the microfluidic device.

15. The method of claim 1, wherein the enzyme comprises a kinase, the enzyme substrate comprises a kinase substrate, and the product comprises a phosphorylated product.

20 16. The method of claim 15, wherein the kinase comprises a protein kinase, a protein kinase A, a protein kinase B, a protein kinase C, a hexokinase, a phosphofructokinase, a phosphoglycerate kinase, a pyruvate kinase, a cyclic AMP-dependent protein kinase, a cyclic GMP-dependent protein kinase, a calmodulin-dependent protein kinase II, a casein kinase I, a casein kinase II, a glycogen synthase  
25 kinase-3, a cyclin-dependent kinase, a p34/cdc2 kinase, or a nucleic acid kinase.

17. The method of claim 1, wherein the enzyme comprises a phosphatase, the enzyme substrate comprises a phosphatase substrate, and the product comprises a dephosphorylated product.

18. The method of claim 17, wherein the phosphatase comprises a protein phosphatase, an acid phosphatase, an alkaline phosphatase, a sugar phosphatase, or a polynucleotide phosphatase.

19. The method of claim 1, wherein prior to the flowing step, the method comprises:

flowing at least the enzyme through a first channel in fluid communication with an enzyme source into a mixing region of the microfluidic device; and,

flowing at least the enzyme substrate through a second channel in fluid communication with an enzyme substrate source into the mixing region, wherein the enzyme converts at least some of the enzyme substrate to the product, thereby producing the reaction mixture.

20. The method of claim 19, wherein a microchannel comprises the mixing region.

21. The method of claim 1, the method further comprising flowing the ion-exchange material into the separation region.

22. The method of claim 21, wherein the flowed ion-exchange material coats an inner surface of the separation region.

23. The method of claim 21, comprising continuously flowing the ion-exchange material into the separation region for a selected period of time.

24. The method of claim 21, comprising flowing multiple aliquots of the ion-exchange material into the separation region.

25. The method of claim 21, wherein the ion-exchange material is stored in a reservoir, which reservoir is in fluid communication with the separation region.

26. The method of claim 21, comprising flowing one or more other chromatographic materials or surface coatings into the separation region.

27. The method of claim 26, comprising flowing the ion-exchange material and the other chromatographic materials or surface coatings sequentially into the separation region.

28. The method of claim 27, wherein each sequentially flowed material or surface coating coats or modifies an inner surface of the separation region or a previously flowed material which coats the inner surface of the separation region.

29. The method of claim 1, the flowing step further comprising flowing one or more eluents or separation buffers into the separation region from one or more microchannels in fluid communication with the separation region.

30. The method of claim 29, further comprising varying a concentration of the one or more eluents or separation buffers flowed into the separation region to control separation of materials within the separation region.

31. The method of claim 1, further comprising sampling the enzyme, the enzyme substrate, and/or an additional material from one or more sources external to the microfluidic device.

32. The method of claim 31, wherein the additional material comprises one or more of: a modulator, an inhibitor, an antagonist, an agonist, an eluent, or a separation buffer.

33. The method of claim 31, wherein the one or more sources are present in a microtiter dish and wherein the microfluidic device comprises one or more external capillary elements in fluid communication with the separation region, the method comprising contacting the one or more external capillary elements to the one or more sources and drawing fluid out of the one or more sources, into the one or more external capillary elements, and into the microfluidic device.

**34.** A method of performing a mobility shift assay in a microfluidic device, the method comprising:

flowing a mixture comprising at least first and second materials through a separation region of the microfluidic device under an applied pressure, which separation region comprises an amphiphilic material, whereby the first and second materials separate from one another in the separation region to produce separated first and second materials; and,

detecting at least one of the separated first and second materials, thereby performing the mobility shift assay in the microfluidic device.

**35.** The method of claim 34, wherein the first and second materials are separated based upon distinguishing amphiphilic properties.

**36.** The method of claim 34, wherein the first and second materials independently comprise: a biological molecule, an artificial molecule, an ion, a polar molecule, an apolar molecule, an antibody, an antigen, an inorganic molecule, an organic molecule, a drug, a receptor, a ligand, a neurotransmitter, a cytokine, a chemokine, a hormone, a particle, a bead, a functionalized bead, a liposome, a cell, a nucleic acid, DNA, RNA, an oligonucleotide, a ribozyme, a protein, a phosphoprotein, a glycoprotein, a lipoprotein, a peptide, a phosphopeptide, a glycopeptide, a lipopeptide, an enzyme, an enzyme substrate, a product, a carbohydrate, a lipid, a label, a dye, or a fluorophore.

**37.** The method of claim 34, wherein the first and/or second material comprises a label.

**38.** The method of claim 34, wherein the first and second materials comprise different net charges in solution.

**39.** The method of claim 34, wherein the mixture comprises at least a third material, which third material separates from the first and second materials in the separation region.

**40.** The method of claim 34, wherein at least the mixture is flowed in the microfluidic device in an absence of an applied electric field.

41. The method of claim 34, wherein at least the mixture is flowed in the microfluidic device under at least one simultaneously applied electric field.

42. The method of claim 34, wherein a microchannel comprises the separation region.

5 43. The method of claim 34, wherein the applied pressure is produced by a vacuum pump operably connected to the microfluidic device through a port that fluidly communicates with the separation region.

44. The method of claim 34, wherein the detecting step comprises at least an optical, a spectroscopic, a fluorescent, a mass, or a luminescent detection.

10 45. The method of claim 34, wherein a plurality of microbeads or a gel comprises the amphiphilic material.

46. The method of claim 34, wherein an inner surface of the separation region comprises the amphiphilic material.

15 47. The method of claim 34, wherein the amphiphilic material is coated on an inner surface of the separation region.

48. The method of claim 34, wherein the first and second materials are mixed in a mixing region of the microfluidic device to produce the mixture prior to flowing through the separation region.

20 49. The method of claim 48, wherein a microchannel comprises the mixing region.

50. The method of claim 34, the method further comprising flowing the amphiphilic material into the separation region.

51. The method of claim 50, wherein the flowed amphiphilic material coats an inner surface of the separation region.

52. The method of claim 50, comprising continuously flowing the amphiphilic material into the separation region for a selected period of time.

53. The method of claim 50, comprising flowing multiple aliquots of the amphiphilic material into the separation region.

5 54. The method of claim 50, wherein the amphiphilic material is stored in a reservoir, which reservoir is in fluid communication with the separation region.

55. The method of claim 50, comprising flowing one or more other chromatographic materials or surface coatings into the separation region.

10 56. The method of claim 55, comprising flowing the amphiphilic material and the other chromatographic materials or surface coatings sequentially into the separation region.

57. The method of claim 56, wherein each sequentially flowed material or surface coating coats or modifies an inner surface of the separation region or a previously flowed material which coats the inner surface of the separation region.

15 58. The method of claim 34, the flowing step further comprising flowing one or more eluents or separation buffers into the separation region from one or more microchannels in fluid communication with the separation region.

20 59. The method of claim 58, further comprising varying a concentration of the one or more eluents or separation buffers flowed into the separation region to control separation of the first and second materials within the separation region.

60. The method of claim 34, further comprising sampling the first material, the second material, and/or an additional material from one or more sources external to the microfluidic device.

25 61. The method of claim 60, wherein the additional material comprises one or more of: a modulator, an inhibitor, an antagonist, an agonist, an eluent, or a separation buffer.

62. The method of claim 60, wherein the one or more sources are present in a microtiter dish and wherein the microfluidic device comprises one or more external capillary elements in fluid communication with the separation region, the method comprising contacting the one or more external capillary elements to the one or more sources and drawing fluid out of the one or more sources, into the one or more external capillary elements, and into the microfluidic device.